

Growth curve with OD_{600} measurements (v1)

Materials:

- Optical neutral cuvettes (1 mL capacity)
- Spectrophotometers
- LB broth
 - with with ampicillin 100 $\mu\text{g/mL}$
 - with chloramphenicol 37 $\mu\text{g/mL}$
 - with both antibiotics
- 250 mL flasks

Procedure:

1. Prepare 10 mL liquid culture in 12 mL round-bottom tubes and incubate them overnight (ON) at 37 °C, 220 rpm.
2. The day after dilute 10-times the liquid culture in 50 mL fresh LB broth (250 mL flasks are used) supplied with proper antibiotic(s) and measure the Optical density at $\lambda = 600 \text{ nm}$ (OD_{600}). The OD_{600} should be between 0.1 and 0.5, where the relationship between OD and cell density is linear.
3. Incubate the newly inoculated culture at 37 °C, 220 rpm.
4. Every 30 min, after vigorous mixing, transfer 1 mL of cell in optical neutral cuvettes. Do NOT label or touch the cuvettes' smooth side. To keep track of the samples, label the cuvettes holder and move only one cuvette at a time. Immediately put the flasks back in the incubator.
5. Set the spectrophotometer on absorbance (Asb) and the wavelength (λ) at 600 nm.
6. Mix the sample in the cuvettes by pipetting up and down and measure the OD_{600} using LB-AMP-CAM broth as blank.
7. Repeat from step 4 till $OD_{600} \approx 0.6$. At $OD_{600} \approx 0.6$ the cell are induced.
8. Add 200 ng/mL tetracycline (Tet) to the flasks in the incubator.
9. Place back the flasks at 37 °C, 220 rpm.
10. After the desired incubation time step (30 minutes to 10 hours, depending on the experiment), after vigorous mixing, transfer 1 mL of cell in optical neutral cuvettes as in step 4.
11. Repeat step 10 at appropriate time intervals during the growth experiment.
12. Transfer the OD_{600} measurements to an electronic sheet, calculate the \log_{10} of each data and draw a growth curve.